HLT Method GPA 004

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Cleanup and Analysis of Fish Tissue for Low Level Priority Pollutant Analysis

DRAFT

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1. Scope and Application

This method covers the determination of acid/neutral semivolatile organic compounds that may be present in tissue samples. This method involves the use of gel permeation chromatography and water partitioning as cleanup steps for the sample. Analysis is performed by full scan gas chromatography/mass spectrometry. Analysis of spiked samples have shown good recovery (60 - 90 %) at the 40 ug/kg level.

This method is restricted to use by or under the supervision of analysts experienced in the operation of gel permeation chromatography equipment, the operation of gas chromatography/mass spectrometers and the interpretation of mass spectra. Each analyst using this method must demonstrate acceptable results with this method by the analysis of spiked samples.

2. Summary of Method

A measured sample of tissue (approximately 50 g) is extracted with acetone using a tissue homogenizer. The organic extract is concentrated, exchanged to methylene chloride and dried with anhydrous sodium sulfate. The dried methylene chloride extract is concentrated to approximately 5 ml and loaded into the sample loop of the gel permeation chromatography system using a column packed with Biobeads SX-3 (2000 MW cutoff). A predetermined fraction eluting from the GPC unit that contains the compounds of interest is collected. This collected fraction is concentrated and then placed into approximately 1 liter of water at pH 2 and extracted with methylene chloride. The extract is concentrated to approximately 5 ml and loaded in the sample loop of a GPC system using a column packed with Biobeads SX-8 (1000 MW cutoff). Again a predetermined fraction that contains the compounds of interest is collected. The collected fraction is concentrated to 1 ml and analyzed by GC/MS. The chromatographic conditions permit the separation and determination of the parameters in the extract. Qualitative identification is based upon retention time and mass spectral matching. Quantitation is performed using internal standard methods and a single characteristic ion.

3. Interferences

Method interferences may be caused by contamination in solvents, reagents, glassware and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All materials used must be routinely demonstrated to be free from interferences under the conditions of analysis by running laboratory reagent blanks.

For additional details see EPA Method 625 or EPA Contract Laboratory Program Statement of Work for Organic Analysis.

4. Safety

The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis.

Some of the compounds covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens.

5. Apparatus and Materials

Analytical Balance- readable to 0.001 g.

Tissue Homogenizer with Stainless Steel Blades.
Brinkman Polytron or equivalent.

Glass Centrifuge Bottles- 250 ml capacity.

Kuderna Danish Concentrator with 3 ball Snyder Column.

Boiling chips.

Water Bath for concentrating solvent extracts.

Erlymeyer flasks- 500 ml.

Gel permeation chromatography system - Consisting of a pump capable of delivering at least 5 ml/min, sample injection valve with 10 ml sample loop, glass column with teflon plungers, UV detector (254 nm), automatic switching valves to collect desired fraction, recording device for signal from UV detector (chart recorder or integrator). With separate systems equipped with two separate columns packed with Biobeads SX-3 and SX-8. Additional details may be found in the EPA Contract Laboratory Program Statement of Work Organic Analysis.

Gas Chromatography/Mass Spectrometer- see EPA Method 625 for details. Analysis is performed with a DB-5 0.25mm x 30 m column, or equivalent, inserted into the source of the mass spectrometer.

6. Reagents

Organic free water- Organic free water is defined as water in which no interferences are observed at the method detection limit for each parameter of interest.

Sulfuric acid solution (1+1 vol/vol).

Acetone and Methylene chloride- Pesticide or distilled in glass quality.

Sodium Sulfate- (ACS) Granular, anhydrous. Purify by heating in a muffle furnace at 400 C overnight.

Analytical and surrogate standards - see method 625 for additional details.

7. Calibration

Instrument calibration should be with multiple point calibration curves. See method 625 or the EPA Contract Laboratory Program Statement of Work Organic Analysis for additional details.

8. Quality Control

Good quality control procedures are essential for obtaining meaningful data. All laboratories should have an effective quality control/quality assurance program in place. For additional details see method 625 or the EPA Contract Laboratory Program Statement of Work Organic Analysis .

9. Sample Collection, Preservation and Handling

Tissue samples should be obtained by personnel trained in the collection of tissue samples for trace organic analysis. This will require knowledge of biology so that the proper tissue samples are obtained and knowledge of potential contamination problems arising from trace environmental analysis.

The whole fish should be kept refrigerated until the tissue sub-samples can be obtained. The collected tissue sub-samples should be placed in precleaned glass jar with Teflon lid liners and kept frozen until time of analysis. The frozen sub-samples should be thawed just enough to obtain a representative portion and not be left at room temperature for extended periods of time. Sample holding times for tissues have not been determined. Ideally, the tissue samples should be analyzed as soon as possible after collection. The sample extracts should be kept refrigerated and analyzed within 40 days of extraction.

10. Tissue sample preparation.

1. Weigh approximately 50 g of thawed tissue in a 250 ml centrifuge bottle. Add an appropriate surrogate or standard spiking solutions.
2. Add 100 ml of acetone to the centrifuge bottle. Homogenize the tissue with the tissue homogenizer operating at full speed for 3 minutes.
3. Decant the acetone extract into a funnel containing a Whatman 41 filter paper. Collect the filtered extract in a 500 ml Erlymeyer flask.
4. Extract the tissue two more times by the above procedure. All acetone extracts are combined. After the final extraction transfer the tissue to the filter and rinse with additional acetone.
5. Add a boiling chip to the Erlymeyer flask equipped with a 3 ball Snyder column. Concentrate the acetone extract to approximately 25 ml volume or until it begins to separate into two phases (one phase is water).
6. After the extract has cooled, add approximately 200-250 ml of methylene chloride followed by enough anhydrous sodium sulfate to dry the sample extract. After the extract is dry, transfer it to a Kuderna-Danish (K-D) concentration apparatus. Concentrate the extract to a volume of approximately 5 ml.
7. Load the entire extract into the sample injection loop of the GPC system. Include all rinsings. Pass the sample through the column containing Biobeads SX-3 and collecting the previously determined fraction containing compounds of interest in a K-D apparatus.

The extract loaded on the GPC column should have no more than 0.1g lipids/ ml of extract ~~is recommended~~ for best cleanup performance.

Additional details regarding set up and calibration of the GPC system can be found in the EPA Contract Laboratory Program Statement of Work for Medium and Low Level Organic Analysis.
8. Add a 3 ball Snyder column to the K-D and concentrate the extract to a volume of approximately 5 ml. Allow the extract to cool.
9. Add the extract to approximately ~~1 liter of~~ organic free water, adjust the pH to less than 2 with sulfuric acid and extract the water three times with 100 ml (3 x ~~100ml~~) methylene chloride. Combining all extracts in a K-D and concentrate the extract to approximately 5 ml. Allow extract to cool.
10. Load the extract with solvent rinsings into the sample injection valve of the GPC system equipped with a Biobead SX-8 column. Inject the sample and collect the desired fraction in a K-D apparatus. Place a three ball Snyder column on the K-D and concentrate to approximately 5 ml.
11. Continue concentration of the collected eluant to a volume of 1 ml using the steam bath and nitrogen evaporation bath. The extract is now ready for analysis by GC/MS.
12. The GC/MS scanning parameters are specified in either Method 625 or the CLP SOW. The GC conditions should be optimized for separation of the compounds of interest. Sample injection should be under splitless injection conditions, with the starting column

temperature near 30 C. The injected sample volume is 2 microliters.

11. Daily GC/MS Performance Tests

See EPA Method 625 for details.

12. Gas Chromatography/ Mass Spectrometry

See EPA Method 625 for details.

13. Qualitative Identification

See EPA Method 625 for details.

14. Calculations

See EPA Contract Laboratory Program Statement of Work Organic Analysis for calculations involving solid matrices.

15. Method Performance

The following recoveries have been obtained for replicate analysis of fish tissue by this method. Cleanup method A utilizes the following sequence: GPC Cleanup with Biobeads SX-3, water back extraction, GPC cleanup with Biobeads SX-8. Cleanup method B involves using the following sequence: water back extraction, GPC Cleanup with Biobeads SX-3, GPC cleanup with Biobeads SX-8. The spiking of all priority pollutant target compounds was done at two levels, these being 40 ug/kg and 80 ug/kg.

Compounds	Method A	Method B
Grand Mean	90(41%)a	78(41%)a
(60 compounds)	96(35%)b	87(39%)b
Chlorobenzenes	46(25%)a	39(29%)a
(4 compounds)	66(41%)b	48(28%)b
Phenols	111(47%)a	84(45%)a
(14 compounds)	99(17%)b	90(41%)b
PAHs	97(18%)a	83(20%)a
(22 compounds)	104(27%)b	93(18%)b

(x%) is average relative standard deviation
a- spiked at 80 ug/kg
b- spiked at 40 ug/kg

16. References

1. Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, U.S. Environmental Protection Agency, July 1982, EPA-600/4-82-057.

2. U.S. Environmental Protection Agency, Contract Laboratory Program, Statement of Work, Organic Analysis, February 1988.

3. Stalling, D.L., Tindle, R.C., Johnson, J.L.; Jour. Off. Anal. Chem.; 55(1972), P.32-38.

4. Federal Register, EPA Method 625, 1984.

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Fish Spike Data

Compound	40 Mean 80 ppb	Std. Dev.	% RSD
1,2,4-TRICHLOROBENZENE	74.19	6.72	9.1%
1,2-DICHLOROBENZENE	58.11	14.16	24.4%
1,3-DICHLOROBENZENE	42.09	7.70	18.3%
1,4-DICHLOROBENZENE	51.74	11.66	22.5%
2,4,5-TRICHLOROPHENOL	79.22	18.03	22.8%
2,4,6-TRICHLOROPHENOL	139.84	20.56	14.7%
2,4-DICHLOROPHENOL	90.53	4.94	5.5%
2,4-DIMETHYLPHENOL	129.41	36.63	28.3%
2,4-DINITROTOLUENE	70.09	18.36	26.2%
2,6-DINITROTOLUENE	73.00	11.92	16.3%
2-CHLORONAPHTHALENE	89.92	5.92	6.6%
2-CHLOROPHENOL	72.77	27.84	38.3%
2-METHYLNAPHTHALENE	85.47	5.72	6.7%
2-METHYLPHENOL	110.81	14.81	13.4%
2-NITROPHENOL	66.97	3.99	6.0%
4,6-DINITRO-2-METHYLPHEN	33.88		
4-BROMOPHENYL-PHENYLETHE	105.91	27.99	26.4%
4-CHLOROPHENYL-PHENYLETH	102.16	16.24	15.9%
4-CHLORO-3-METHYLPHENOL	82.31	12.95	15.7%
4-METHYLPHENOL	139.00	37.12	26.7%
ACENAPHTHENE	103.16	8.70	8.4%
ACENAPHTHYLENE	99.50	5.01	5.0%
ANTHRACENE	79.03	24.55	31.1%
BENZO(A)ANTHRACENE	114.88	12.47	10.9%
BENZO(A)PYRENE	103.00	12.47	12.1%
BENZO(B)FLUORANTHENE	106.75	7.08	6.6%
BENZO(G,H,I)PERYLENE	83.91	7.24	8.6%
BENZO(K)FLUORANTHENE	106.75	7.08	6.6%
BIS(2-CHLOROETHOXY)METHA	88.31	6.21	7.0%
BIS(2-CHLOROETHYL)ETHER	67.34	16.81	25.0%
BIS(2-CHLOROISOPROPYL)ET	68.94	11.47	16.6%
BUTYLBENZYLPHTHALATE	156.67	41.27	26.3%
CHRYSENE	89.16	13.37	15.0%
DIBENZOFURAN	94.41	8.91	9.4%
DIBENZ(A,H)ANTHRACENE	83.38	12.85	15.4%
DIETHYLPHTHALATE	115.91	20.79	17.9%
DIMETHYL PHTHALATE	109.84	9.75	8.9%
FLUORANTHENE	88.06	14.38	16.3%
FLUORENE	101.50	7.28	7.2%
HEXACHLOROBENZENE	106.91	19.85	18.6%
HEXACHLOROBUTADIENE	71.06	6.84	9.6%
HEXACHLOROETHANE	44.19	18.69	42.3%
INDENO(1,2,3-CD)PYRENE	81.47	11.43	14.0%
ISOPHORONE	147.91	61.27	41.4%
NAPHTHALENE	81.44	7.19	8.8%
NITROBENZENE	94.89	41.15	43.4%
N-NITROSO-DI-N-PROPYLAMI	70.34	9.66	13.7%
PENTACHLOROPHENOL	214.13	64.40	30.1%
PHENANTHRENE	140.50	21.85	15.6%
PHENOL	89.50	24.06	26.9%
PYRENE	111.88	13.78	12.3%
Ave	94.35	17.02	17.5%
Min	33.88	3.99	5.0%
Max	214.13	64.40	43.4%

Fish Spike Data

Compound	Mean 150 ppb	Std. Dev.	% RSD
1,2,4-TRICHLOROBENZENE	61.56	13.09	21.3%
1,2-DICHLOROBENZENE	41.13	14.96	36.4%
1,3-DICHLOROBENZENE	31.85	17.26	54.2%
1,4-DICHLOROBENZENE	35.58	16.26	45.7%
2,4,5-TRICHLOROPHENOL	108.56	33.73	31.1%
2,4,6-TRICHLOROPHENOL	126.00	7.50	6.0%
2,4-DICHLOROPHENOL	86.63	7.35	8.5%
2,4-DIMETHYLPHENOL	104.06	22.11	21.2%
2,4-DINITROTOLUENE	65.38	22.42	34.3%
2,6-DINITROTOLUENE	75.25	10.37	13.8%
2-CHLORONAPHTHALENE	79.56	6.03	7.6%
2-CHLOROPHENOL	71.50	11.88	16.6%
2-METHYLNAPHTHALENE	80.63	9.96	12.4%
2-METHYLPHENOL	111.88	35.13	31.4%
2-NITROPHENOL	56.88	7.17	12.6%
4,6-DINITRO-2-METHYLPHEN	37.30	14.95	40.1%
4-BROMOPHENYL-PHENYLETHE	80.69	10.97	13.6%
4-CHLOROPHENYL-PHENYLETH	90.44	4.80	5.3%
4-CHLORO-3-METHYLPHENOL	126.19	66.19	52.5%
4-METHYLPHENOL	117.25	37.74	32.2%
ACENAPHTHENE	88.88	4.32	4.9%
ACENAPHTHYLENE	92.06	2.48	2.7%
ANTHRACENE	84.49	40.05	47.4%
BENZO(A)ANTHRACENE	110.44	10.46	9.5%
BENZO(A)PYRENE	92.75	4.61	5.0%
BENZO(B)FLUORANTHENE	93.19	7.30	7.8%
BENZO(G,H,I)PERYLENE	90.94	20.33	22.4%
BENZO(K)FLUORANTHENE	93.92	8.44	9.0%
BIS(2-CHLOROETHOXY)METHA	74.13	7.10	9.6%
BIS(2-CHLOROETHYL)ETHER	51.56	9.01	17.5%
BIS(2-CHLOROISOPROPYL)ET	48.18	15.99	33.2%
BUTYLBENZYLPHTHALATE	109.42	21.64	19.8%
CHRYSENE	88.25	14.99	17.0%
DIBENZOFURAN	88.58	3.40	3.8%
DIBENZ(A,H)ANTHRACENE	98.63	19.99	20.3%
DIETHYLPHTHALATE	105.88	9.83	9.3%
DIMETHYL PHTHALATE	101.94	5.39	5.3%
FLUORANTHENE	101.75	35.08	34.5%
FLUORENE	95.88	6.11	6.4%
HEXACHLOROBENZENE	96.44	11.10	11.5%
HEXACHLOROBUTADIENE	54.98	15.80	28.7%
HEXACHLOROETHANE	30.25	14.32	47.3%
INDENO(1,2,3-CD)PYRENE	93.44	19.14	20.5%
ISOPHORONE	71.94	6.55	9.1%
NAPHTHALENE	69.69	12.61	18.1%
NITROBENZENE	89.25	15.10	16.9%
N-NITROSO-DI-N-PROPYLAMI	61.56	5.58	9.1%
PENTACHLOROPHENOL	172.50	27.00	15.7%
PHENANTHRENE	88.19	27.61	31.3%
PHENOL	76.31	23.49	30.8%
PYRENE	94.06	18.59	19.8%
Ave	84.27	15.95	20.4%
Min	30.25	2.48	2.7%
Max	172.50	66.19	54.2%

Fish Spike Data

400 ~~800~~ ppb

Compound	Mean	Std. Dev.	% RSD
1,2,4-TRICHLOROBENZENE	63.75	4.15	6.5%
1,2-DICHLOROBENZENE	36.00	2.35	6.5%
1,3-DICHLOROBENZENE	24.25	2.17	8.9%
1,4-DICHLOROBENZENE	27.75	2.28	8.2%
2,4,5-TRICHLOROPHENOL	88.75	21.28	24.0%
2,4,6-TRICHLOROPHENOL	100.00	15.67	15.7%
2,4-DICHLOROPHENOL	82.50	7.89	9.6%
2,4-DIMETHYLPHENOL	60.00	35.80	59.7%
2,4-DINITROTOLUENE	76.75	5.54	7.2%
2,6-DINITROTOLUENE	80.50	9.86	12.3%
2-CHLORONAPHTHALENE	81.25	8.70	10.7%
2-CHLOROPHENOL	73.50	6.54	8.9%
2-METHYLNAPHTHALENE	81.50	7.92	9.7%
2-METHYLPHENOL	78.75	19.56	24.8%
2-NITROPHENOL	66.00	7.55	11.4%
4,6-DINITRO-2-METHYLPHEN	62.25	11.71	18.8%
4-BROMOPHENYL-PHENYLETHE	85.50	5.94	6.9%
4-CHLOROPHENYL-PHENYLETH	91.00	5.79	6.4%
4-CHLORO-3-METHYLPHENOL	96.50	12.34	12.8%
4-METHYLPHENOL	95.25	6.53	6.9%
ACENAPHTHENE	82.25	8.50	10.3%
ACENAPHTHYLENE	90.25	14.10	15.6%
ANTHRACENE	72.25	22.25	30.8%
BENZO(A)ANTHRACENE	103.25	5.63	5.5%
BENZO(A)PYRENE	86.75	10.16	11.7%
BENZO(B)FLUORANTHENE	92.75	13.37	14.4%
BENZO(G,H,I)PERYLENE	72.75	16.84	23.2%
BENZO(K)FLUORANTHENE	92.50	12.97	14.0%
BIS(2-CHLOROETHOXY)METHA	67.75	9.91	14.6%
BIS(2-CHLOROETHYL)ETHER	45.75	4.49	9.8%
BIS(2-CHLOROISOPROPYL)ET	48.75	10.03	20.6%
BUTYLBENZYLPHTHALATE	92.25	42.63	46.2%
CHRYSENE	90.75	13.55	14.9%
DIBENZOFURAN	86.75	7.66	8.8%
DIBENZ(A,H)ANTHRACENE	77.75	13.05	16.8%
DIETHYLPHTHALATE	94.25	6.22	6.6%
DIMETHYL PHTHALATE	91.50	1.50	1.6%
FLUORANTHENE	92.75	8.64	9.3%
FLUORENE	91.00	9.25	10.2%
HEXACHLOROBENZENE	92.25	10.03	10.9%
HEXACHLOROBUTADIENE	51.00	5.79	11.3%
HEXACHLOROETHANE	22.50	2.50	11.1%
INDENO(1,2,3-CD)PYRENE	78.25	12.97	16.6%
ISOPHORONE	65.25	10.57	16.2%
NAPHTHALENE	66.50	6.87	10.3%
NITROBENZENE	55.25	7.53	13.6%
N-NITROSO-DI-N-PROPYLAMI	58.75	6.76	11.5%
PENTACHLOROPHENOL	160.00	37.42	23.4%
PHENANTHRENE	93.50	5.72	6.1%
PHENOL	71.00	12.14	17.1%
PYRENE	93.00	13.36	14.4%
Ave	77.08	11.02	14.2%
Min	22.50	1.50	1.6%
Max	160.00	42.63	59.7%